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Hemocyte populations in *Zonocerus variegatus* (L.) (Orthoptera: Pyrgomorphidae) during post-embryonic development

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Abstract: The focus of this study is to examine the trend in the number and types of hemocytes in all the developmental stages of *Zonocerus variegatus*. The types and number of hemocyte cells present in *Z. variegatus* during post-embryonic development was evaluated with the aid of hemacytometer. Six hemocyte cells were observed in all the developmental stages from the 1st instar larva to the adult stage, namely: prohaemocytes (PRS), plasmatocytes (PLS), granulocytes (GRS), sherulocytes (SPS), oenocytes (OES) and adipohaemocytes (ADS). However, OES was not found in the haemolyph of 1st instar larval stage. PLS had the highest total mean count while OES had the least total mean count of all the six hemocytes. The adult stage had significantly ($P < 0.05$) higher hemocyte count relative to other developmental stages, however, no significant difference ($P > 0.05$) existed between the hemocyte count of the 1st and the 2nd instar larval stages. This study shows that the adult stage is immununilogically able to adapt to the environment better than other lower developmental stages.

Key words: *Zonocerus variegatus*; hemocyte cells; post-embryonic development; hemocyte count; hemacytometer

1 INTRODUCTION

The African variegated grasshopper, *Zonocerus variegatus* (L.) is a tropical African grasshopper and occurs in relatively warm climate (Youdeowei, 1974). It has two annual populations: wet season population (April - August) and dry season population (January - March) (Toye, 1971), however these two annual populations do not interbreed.

The insect life cycle is built around six nymphal stages and an annual stage (Toye, 1971; Ademolu, 2008). The structures and complexity of the insect increases as the insect moult from the 1st instar larval stage to the adult stage and this is more pronounced between the 6th instar larval stage and adult stage (Ademolu *et al.*, 2006)

Studies have shown that the early stages show preference for *Chromoelana odorata*, while the later instars (4th instar larva - adult) prefer *Manihot esculenta*. The physiology/biology of this insect varies from one developmental stage to another, for instance the haemolymph pH of the adult *Z. variegatus* ranged from 6.0 - 6.5, while that of 6th instar larva was between 6.5 and 6.9 (Idowu and Modder, 1998). Similarly, Ademolu *et al.* (2007) observed that the adult stage of *Z. variegatus* had significantly higher ($P < 0.05$) concentration of tissues (fat body, haemolymph and femoral muscles) metabolites than the lower instars. Mullins (1985) also reported that the numbers of hemocytes during circulation vary depending on the physiological state of the insect. In *Z. variegatus*, however, only the adult stage has been investigated (Idowu and Sonde, 2004) as far as hemocyte types are concerned, thus the focus of this study is to assess the types and number of hemocyte cells in *Z. variegatus* during post-embryonic development.

2 MATERIALS AND METHODS

2.1 Insect collection and maintenance

Newly-hatched 1st instar nymphs were collected from their oviposition sites around an uncultivated farm land on the Campus of University of Agriculture, Abeokuta, Ogun State between September and October, 2006. They were maintained on *C. odorata* and *M. esculenta* leaves in the insectary of the Department of Biological Sciences, University of Agriculture, Abeokuta, Nigeria in a wire cage (30 cm × 30 cm × 15 cm) at the temperature of 28 - 31℃ and a relative humidity of 64% - 70%.

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2.2 Estimation of numbers of hemocyte cells

The haemolymph used for hemocyte estimation was collected by inserting a micro needle into the mid-thoracic junction of the insect. Haemolymph that dripped freely into the needle was collected into a calibrated syringe. The haemolymph from ten individuals was diluted with a buffer solution (1 L hemolymph added to 5 L 0.1 mol/L phosphate buffer).

20 L of the diluted haemolymph collected above was applied to the hemacytometer. With the aid of a compound microscope, the cells that appear within the counting zones were counted. During counting only those that lie within each of sixteen individual square and those touching the upper and right hand lines of the square were considered.

After counting, the following formula (Miller and Stanley, 2000) was used to determine the number of cells per mL of haemolymph:

$$\text{Number of hemocytes/mL} = \frac{\text{Number of hemocytes} \times \text{dilution factor} \times 10^5}{\text{Number of zones counted}}$$

2.3 Data statistics and analysis

The experiment was replicated thrice using ten individuals per instar. All data collected were subjected to analysis of variance (ANOVA) (Steel and Torrie, 1980) and significantly different means were separated using Duncan's multiple range test (Duncan, 1955).

3 RESULTS

Six haemocytes were observed in all the stages of *Z. variegatus* and these are: prohaemocytes (PRS), plasmotocytes (PLS), granulocytes (GRS), spherulocytes (SPS), oenocytes (OES) and adipohaemocytes (ADS), however, OES was not found in the haemolymph of 1st instar larva. PLS had the highest total mean count while OES had the least total number of all the six haemocytes. The adult stage had significantly higher haemocyte count ($P < 0.05$) relative to other developmental stages (Table 1); however, no significant difference ($P > 0.05$) existed between the hemocyte counts of the 1st and the 2nd instar larvae. Correlation analysis between the age and the hemocytes cells (Table 2) revealed a positive and strong correlation (except oenocytes).

Table 1 Mean count of the hemocyte of *Zonocerus variegatus* during post-embryonic development ($\times 10^5$ per mL of haemolymph)

Growth stages	PLS	GRS	SPS	OES	ADS	PRS	Total
1st instar larva	4.00 ± 0.05 c	3.00 ± 0.50 b	3.75 ± 0.05 b	–	1.50 ± 0.10 c	4.50 ± 0.20 e	16.75 d
2nd instar larva	3.50 ± 0.01 c	2.00 ± 0.10 b	3.75 ± 0.50 b	7.50 ± 0.10 a	2.50 ± 0.50 c	3.75 ± 0.10 f	14.00 d
3rd instar larva	7.50 ± 0.75 bc	6.50 ± 0.10 ab	8.75 ± 0.05 ab	2.75 ± 0.01 b	3.50 ± 1.10 c	8.25 ± 0.50 d	37.25 c
4th instar larva	10.50 ± 0.20 ab	8.25 ± 0.25 a	12.50 ± 0.50 a	1.25 ± 0.25 b	5.25 ± 0.50 bc	9.25 ± 0.01 c	47.01 b
5th instar larva	10.20 ± 0.20 ab	8.75 ± 0.25 a	9.25 ± 0.75 ab	2.50 ± 0.70 b	8.50 ± 0.50 ab	11.00 ± 0.40 b	50.20 b
6th instar larva	11.00 ± 2.10 a	8.50 ± 0.20 a	9.00 ± 3.60 ab	3.50 ± 0.50 ab	9.00 ± 1.50 ab	7.78 ± 0.02 d	48.75 b
Adult	13.70 ± 3.00 a	10.00 ± 3.50 a	12.00 ± 1.80 a	3.50 ± 2.10 ab	12.00 ± 0.10 a	11.75 ± 0.25 a	62.75 a
Total	59.90	47.00	59.00	21.01	42.25	56.25	

PLS: Plasmatocytes; GRS: Granulocytes; SPS: Spherulocytes; OES: Oenocytes; ADS: Adipohaemocytes; PRS: Prohaemocytes. Mean values in the same column having different superscripts are significantly different ($P < 0.05$, DMRT).

Table 2 The correlation analysis between the age and the hemocyte cells of *Zonocerus variegatus* (L.) during post embryonic development

Hemocyte cells	r^2 values	Significance
Plasmocytes	0.91	*
Granulocytes	0.83	*
Sperulocytes	0.62	*
Oenocytes	0.01	n. s
Adipohaemocytes	0.97	*
Prohaemocytes	0.67	*

* Significant at 99% ($P < 0.01$).

4 DISCUSSION

Six haemocyte types were present in haemolymph of *Z. variegatus* throughout the post embryonic stages, namely: prohaemocyte, plasmatocyte, granulocyte, spherulocyte, oenocyte and adipohaemocyte. Gupta (1985) describe seven basic types of hemocytes common among insects, that is, the six mentioned above and coagulocytes. According to Arnold (1974), coagulocytes are very fragile cells that are not always seen with simple microscope and that might account for their absence

in this study.

Silva *et al.* (2002) reported six haemocytes for *Anastrpha oblique* (Diptera: Tephritidae) and George (2003) reported the same number for infected and non-infected *Boophilus decoloratus* (Koch). Recently, Idowu and Sonde (2004) also found six haemocyte types in the adult of *Z. variegatus* fed different food plants.

Plasmatocytes were the most abundant, followed by prohaemocytes in the haemolymph of *Z. variegatus* during post-embryonic development. The plasmatocytes and prohaemocytes play vital roles in defense and immunity of insects. Plasmatocytes are primarily phagocytic in function but they are also important in wound healing (Gouli *et al.*, 2000). Oenocytes on the other hand were extremely rare and found very few in *Adelges tsugae* Annand (Hom., Adelgidae) (Gouli *et al.*, 2000). The function of oenocytes is unknown but there is information that they have phenol-oxidase which has a role in the metabolism of melanin (Brehelin and Zachery, 1986).

The mean number of hemocyte cells increased during developmental stages from 1st instar larva to adult stage. The increase in the hemocyte number in the haemolymph of insects is a normal response to bacteria or parasite infection (Eslin and Provost, 1998). It was reported earlier that 1st – 3rd instars larval stages are gregarious while the later instars disperse widely, thus, mixing well with immediate environment thereby likely exposed to infection. Similarly, the increased consumption of food plants during post-embryonic development (Idowu and Edema, 2003) might be responsible for increase in infection and thus increased cellular response as evidenced by the higher number of hemocyte cells. Therefore, the increase in number of hemocyte cells of *Z. variegatus* experienced during post-embryonic development indicates that the host defense system was activated as opined by Nappi (1981). This study confirms the susceptibility of the early instar larval stages of *Z. variegatus* and thus their easy elimination as suggested earlier by Toye (1971) and Ademolu *et al.* (2006).

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胚后发育期臭腹腺蝗中的血细胞种类

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摘要: 应用血球计数器统计了胚后发育期臭腹腺蝗 *Zonocerus variegatus* 中存在的血细胞类型和数目。从 1 龄幼虫至成虫的发育阶段中共观察到 6 种血细胞类型，即原血细胞 (PRS)、浆血细胞 (PLS)、粒细胞 (GRS)、珠血细胞 (SPS)、绛色细胞(OES) 和 adipohaemocytes (ADS)。不过，在 1 龄幼虫期未发现 OES。在这 6 种血细胞中，PLS 的总平均数最高，OES 的总平均数最低。成虫期的血细胞数目显著高于其他发育阶段($P<0.05$)，而 1 龄幼虫和 2 龄幼虫期的血细胞数目不存在显著差异($P>0.05$)。

关键词: 臭腹腺蝗; 血细胞; 胚后发育; 血细胞数量; 血球计数器

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